



## THE FIBROUS CAP: A PROMISING TARGET IN THE PHARMACOTHERAPY OF ATHEROSCLEROSIS

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*Recent advances have shed light on the relationship between smooth muscle cell (SMC) phenotypic modulation, resolution of inflammation and atherosclerotic plaque stability. The thick fibrous cap covering the lipid core of plaques is composed of bundles of SMC and collagen fibers and few macrophages and lymphocytes, all of which make the plaque resistant to rupture. The thin fibrous cap contains many macrophages and lymphocytes, few SMC and less collagen fibers, all of which may weaken the cap, leaving the plaque vulnerable to rupture. In the present Dance Round, we, at a pharmacotherapeutic level, address the possibility of how the control over the activity of the essential cellular components of the plaque, particularly its fibrous cap, could be implicated in plaque stabilization, focusing on (i) the modulation of SMC from contractile to secretory (fibrogenic) phenotype, (ii) the control on plaque inflammation-resolution processes, and (iii) the reduction of plaque lipid content. Further studies on both unstable plaque and aortic aneurysm, which share a similar, matrix-based vulnerability, may bring new insights for pharmacotherapy of vascular injuries. **Biomed Rev 2019; 30:136-141***

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*The new paradigm should be not only different but also better.*

**Thomas S. Kuhn,**

*The Structure of Scientific Revolutions*, 1932

In 2017 *Dance Round* one of us together with his colleagues (1) described the following chronology in the ideogenesis of atherosclerosis:

- (i) during the 1960's the great lady of atherosclerosis research Maria Daria Haust proposed the first paradigm shift in the field pointing to a new, synthetic *versus* contractile function of the artery smooth muscle cells (SMC);
- (ii) in the 1970's, we published the first transmission electron microscopic data of this new function conceptualizing it as "secretory function" instead of "synthetic function" of SMC as has been previously considered (reviewed in (2, 3);
- (iii) in the 1980's, atherosclerosis was rediscovered as an inflammatory disease (4);
- (iv) in the 1990's and 2000's, the next paradigm shift has emerged, namely from the inhibition of SMC proliferation (4) and secretion (5, 6) to the stimulation of these same processes aimed at the plaque stabilization by increasing fibrous cap thickness. Briefly, the increased secretion of matrix molecules, particularly (pro)collagen, by SMC could "shift" them from enemies to friends in the fight against the unstable atherosclerotic plaque (1, 7-9).

Yet, it is not clear why fibrous cap destruction takes place in macrophage-rich shoulder regions of the atherosclerotic plaque. Impaired cap formation caused by SMC senescence and/or proneness to apoptosis may also be critical in promoting plaque rupture.

In the present *Dance Round*, we, at a pharmacotherapeutic level, focus on the fibrous cap stabilization targeting (i) the stimulation of SMC secretory (fibrogenic) and proliferative activity, (ii) the inhibition of rupturogenic potential of macrophages and lymphocytes, and (iii) the reduction of atheroma (plaque lipid core). The plaque regression, resulting in the overall reduction in plaque volume (10, 11), is out of scope of the present *Dance Round*.

Of note, keeping in mind the importance of fibrous cap for the vulnerability of the plaque, we must proceed cautiously

with the colchicine administration for cardiovascular diseases (5, 12 and references therein), which antifibrotic action could decrease the thickness of fibrous cap making it vulnerable to erosion and/or rupture.

Atherosclerosis is a low-grade inflammatory disease characterized by occlusion of large- and medium-sized arteries due to the formation of plaques (4). Plaque instability has been associated with erosion, ulceration or rupture of the fibrous cap that covers plaque lipid core. Recent advances have shed light on the relationship between SMC phenotypic modulation, inflammation-resolution and plaque vulnerability-stability (14-17).

### FIBROUS CAP COMPOSITION

The thick fibrous cap covering the lipid core of atherosclerotic plaques is composed of bundles of SMC (and myofibroblasts?) and collagen fibers and few macrophages and lymphocytes. The thin fibrous cap contains many macrophages and lymphocytes, few SMC and less collagen fibers, all of which may weaken the cap, leaving the plaque vulnerable to rupture. This tends to occur in areas where the cap is the thinnest and most heavily infiltrated by macrophages. These are plaque shoulder region defined as the junction between the plaque and the adjacent, less diseased artery wall (Table 1, Fig. 1). In advanced atherosclerotic lesions calcification of the fibrous cap can develop.

**Table 1.** Major features of unstable atherosclerotic plaque

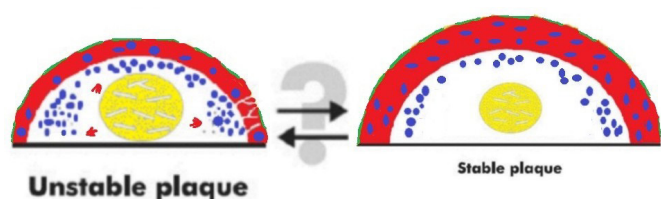
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Thin fibrous cap (< 100 micrometer)
Increased presence of macrophages and lymphocytes
Low presence of SMC and collagen fibers
Large lipid core (> 30% of the total volume of plaque)
Presence of intraplaque hemorrhage
Cap erosion and thrombus formation
Increased expression of matrix degrading MMP
Reduced expression of tissue inhibitors of MMP (TIMP)

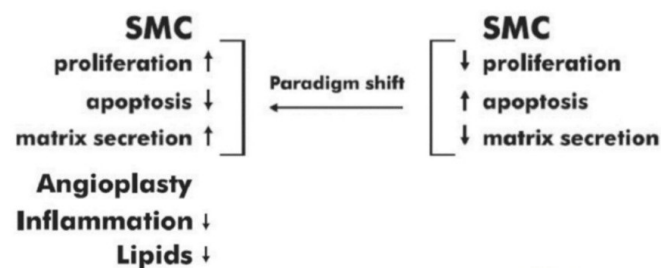
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\*SMC, smooth muscle cells; MMP, Matrix metalloproteinases  
TIMP: Tissue inhibitors of metalloproteinases

To recap, the paradigm was shifted from the plaque size to the plaque stability, that is, the thickness of fibrous cap - the thicker the cap, the more stable the plaque. Accordingly, plaque stabilization has appeared as a promising therapeutic approach for the therapy of atherosclerosis (Fig. 2).



**Figure 1.** A drawing illustrating the composition of unstable and stable atherosclerotic plaque and its possible reversibility (two arrows). Both plaques are covered by single layer of endothelial cells (green) and fibrous caps (red). Unstable plaque has thin fibrous cap containing macrophages (blue granules) and few smooth muscle cells (SMC) (blue spindles), which may weaken the cap, leaving it vulnerable to rupture (white lines in cap's shoulder (at right)). In contrary, stable plaque has thick fibrous cap composed of many SMC (blue spindles) and few macrophages (blue granules). Within the plaque, lipid core (yellow) is larger in unstable than in stable plaque, whereas macrophages and lymphocytes (blue granules) are more in unstable than in stable plaque. In unstable plaque, two intraplaque hemorrhages (red) are also depicted. From (13), a colored version of these latter authors' Figure 1.



**Figure 2.** An illustration of the paradigm shift in the process of atherogenesis (long arrow), SMC, smooth muscle cells. From (13).

### FACTORS INVOLVED IN PLAQUE STABILIZATION

Possible ways for the stabilization of fibrous cap might be: (i) making the cap more fibrous, (ii) attenuating inflammation, stimulating the resolution of inflammation, and (iii) reducing plaque lipid content.

#### Phenotypic modulation of SMC

Traditionally, the conversion from contractile to secretory phenotype of SMC was thought to play a pivotal role in the pathobiology of atherosclerosis (1, 4-6). However, the

molecular aspects of this phenomenon as related to plaque stability are not fully defined. Recently, it was demonstrated that a signaling cross-talk between fibroblast growth factor (FGF) and transforming growth factor-beta (TGF- $\beta$ ) in SMC plays an important role in the atherogenesis, namely, the inhibition of FGF signaling increases TGF- $\beta$  activity, resulting in a significant increase of fibrous cap thickness and decrease of lipid core size (18).

Likewise, the critical role of SMC phenotypic modulation in aortic aneurysm and dissection in Marfan syndrome is intensively studied (19-23). Note, both unstable (thin) fibrous cap and aortic aneurysm share a similar, matrix-based vulnerability.

#### Metalloproteinases inhibitors

Since metalloproteinases (MMP) favor matrix (collagen, elastin, and proteoglycans) degradation, the decrease of MMP activity may have a stabilizing action on the fibrous cap (24-27): In this vein, doxycycline, a MMP-2 and MMP-9 inhibitor, significantly delays thoracic aorta aneurysm rupture in Marfan syndrome-like mice (19, 20). Because of the safety profile of the tetracyclines, clinical trials should be organized to determine if doxycycline could also delay the progression of plaque instability. Another MMP-related example is the phosphinic peptide (RXP470.1) which is a selective murine MMP-12 inhibitor (28).

#### Specialized pro-resolving mediators

Defective resolution of inflammation is the underlying cause of various chronic inflammatory diseases, including atherosclerosis and other cardiometabolic diseases. Inflammation-resolution are active processes aimed at restoration of tissue integrity and function. These are governed in part by endogenously synthesized pro-resolving mediators, such as lipoxins, resolvins, protectins, and maresins. Compromised inflammation-resolution is an important driving force in the progression of atherosclerosis and, respectively, a promising pharmacological target for its therapy (29). In effect, the "resolution pharmacology" might be a therapeutic option for atherosclerosis (30).

Omega-3 essential fatty acids serve as substrates for the formation of a group of lipid-derived mediators that participate in the resolution of inflammation. Among them, the eicosapentaenoic acid (EPA)-derived resolvin E1 appears to be of particular interest, as dietary supplemented EPA was found to preferentially be distributed in thin fibrous cap, implying an important plaque stabilizing potential (31). The direct re-

petitive delivery of pro-resolving mediators themselves also suggests a potentially innovative strategy for resolving arterial inflammation. The systemic administration of resolvin D2 and maresin 1 has been shown in a mice model of atherosclerosis to prevent the expansion of the necrotic core and the accumulation of macrophages, simultaneously increasing the number of SMC and the fibrous cap thickness (32).

### **Acetylcholine anti-inflammatory pathway**

The cholinergic anti-inflammatory pathway is dependent on the nicotinic acetylcholine receptor  $\alpha 7$  subunit. Accordingly, selective acetylcholine receptor  $\alpha 7$  agonists reduce the level of pro-inflammatory mediators (e.g. TNF- $\alpha$ ) in experimental models of inflammatory disease (33). Notably, as reported by Rosso *et al* in this volume of *Biomedical Reviews*, vagus nerve stimulation leads to the increased expression of nerve growth factor (NGF), the latter being also a subject of discussion in the present *Dance Round* (see next section and Table 2),

Further studies on the cholinergic anti-inflammatory mechanisms may provide new insights for the pathogenesis and therapy of atherosclerosis.

### **Nerve growth factor**

In addition to platelet-derived growth factor, FGF and other fibrogenic factors (for galectin-3 see Kisheva and Yotov in this volume of *Biomedical Reviews*), recent studies have demonstrated that nerve growth factor (NGF) has effects on myofibroblasts and collagen synthesis, and, respectively, fibrotic disorders (34, 35).

Keeping in mind that (i) atherosclerotic plaque may be viewed as a vascular wound (1, 4), (ii) topical application of NGF expresses healing effects on corneal ulcers, diabetic wounds, pressure ulcers, and chronic vasculitic ulcers (36-38), and (iii) reduced local and circulating level of NGF was found in human coronary atherosclerosis and metabolic syndrome (39), we suggest that NGF may also demonstrate healing effect on atherosclerotic plaque (vascular wound).

Noteworthy, Professor Russell Ross (1929-1999), the author of more than 300 peer-reviewed papers including the seminal review cited herein (4), has started his research career on cell biology of skin wound before became one of the most renowned scientists in the field of atherosclerosis.

A summing-up list of agents with potentials for plaque stabilization is shown in Table 2.

**Table 2.** Selected list of possible plaque stabilizers

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- Stimulators of matrix molecule secretion by smooth muscle cells (SMC)\*
  - Matrix metalloproteinases-targeting agents
    - Inhibitors of matrix metalloproteinases
    - Activators of tissue inhibitors of metalloproteinases
  - Inflammation-targeting agents
    - Inhibitors of inflammation
    - Stimulators of resolution of inflammation
  - Acetylcholine-targeting agents
    - Nicotinic acetylcholine receptor  $\alpha 7$  agonists
  - Lipid-targeting agents
    - Inhibitors of phosphatidylcholine phospholipase C (40)
      - The xanthate selective inhibitor D609 (41); new selective inhibitors (chiral derivatives of tetrahydrobenzoxazepines) (42)
    - Inhibitors of sphingomyelin synthase 2
      - D609 (43); Ly93 (2-benzyloxybenzamides analog) (44)
    - Statins (10)
    - PCSK-9 (pro-protein convertase subtilisin/kexin type 9) inhibitors: evolocumab, alirocumab (45, 46)
  - NGF and TrkA<sup>NGF</sup> receptor agonists (3)
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\* For SMC apoptosis and proliferation, also lipids, see Fig. 2. For decrease of macrophage proliferation, see (47).

## **EPILOGUE**

Rapidly accelerating knowledge and continued research promise to provide further progress in the prevention and therapy of cardiovascular disease. Atherosclerotic plaque is like a volcano that sleeps - if it erupts, its lava stops the blood flow and the oxygen-deprived heart becomes numb - the silence of the heart is called myocardial infarction.

- Vulnerable plaque
- Vulnerable blood
- Vulnerable myocardium
- Vulnerable patient
- Vulnerable society

## **CONFLICT OF INTEREST**

The authors have no conflict of interest to disclose.

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